

Review

Th2 cytokines and asthma**The role of interleukin-5 in allergic eosinophilic disease**

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Abstract

Interleukin-5 is produced by a number of cell types, and is responsible for the maturation and release of eosinophils in the bone marrow. In humans, interleukin-5 is a very selective cytokine as a result of the restricted expression of the interleukin-5 receptor on eosinophils and basophils. Eosinophils are a prominent feature in the pulmonary inflammation that is associated with allergic airway diseases, suggesting that inhibition of interleukin-5 is a viable treatment. The present review addresses the data that relate interleukin-5 to pulmonary inflammation and function in animal models, and the use of neutralizing anti-interleukin-5 monoclonal antibodies for the treatment of asthma in humans.

Keywords: allergy, asthma, eosinophil, interleukin-5

Introduction

Several allergic diseases, such as nasal rhinitis, nasal polyps, asthma, idiopathic eosinophilic syndromes, and atopic dermatitis, have prominent inflammatory components that are characterized by pronounced eosinophilic infiltration [1]. As a result, the role of chronic pulmonary inflammation in the pathophysiology of asthma has been studied extensively in human and in animal models. In asthma, pulmonary inflammation is characterized by edema, decreased mucociliary clearance, epithelial damage, increased neuronal responsiveness, and bronchoalveolar eosinophilia [1].

Eosinophils form in the bone marrow from myeloid precursors in response to cytokine activation, and are released into the circulation following an appropriate stimulus [2]. Once in the circulation they accumulate rapidly in tissue, where they synthesize and release lipid mediators that can cause edema, bronchoconstriction and chemotaxis, and secrete enzymes and proteins that can damage tissue [2].

The eosinophil is therefore an ideal target for selectively inhibiting the tissue damage that accompanies allergic diseases, without inducing the immunosuppressive consequences that can arise from systemic use of pleiotropic drugs such as steroids.

Interleukin-5 acts as a homodimer, and is essential for maturation of eosinophils in the bone marrow and their release into the blood [3–6]. In humans, interleukin-5 acts only on eosinophils and basophils, in which it causes maturation, growth, activation, and survival [7,8]. This specificity occurs because only those cells possess the interleukin-5 receptor. The functional high-affinity interleukin-5 receptor (250 pmol/l) is composed of two subunits: an α -subunit that is unique to interleukin-5, and a β_c -subunit that is shared with interleukin-3 and granulocyte macrophage-colony stimulating factor (GM-CSF) [9,10].

In animals and in humans, inhibiting interleukin-5 with monoclonal antibodies can reduce blood and broncho-

alveolar eosinophilia caused by allergic challenge or chronic disease [11–14]. Therefore, exclusively inhibiting the actions of interleukin-5 can suppress at least one of the alleged causes of asthma, namely tissue damage due to eosinophil accumulation during pulmonary inflammation.

Although a relationship exists between pulmonary eosinophilia and asthma in humans [15,16], the correlation in animal models between airway hyperreactivity and eosinophilia is less convincing [13,17,18]. However, selective inhibition of interleukin-5 by antibodies can block hyperreactivity in nonhuman primates [14]. Much of the same biology is evident in interleukin-5-knockout mice [19]. Although these mice can produce constitutive levels of eosinophils, they do not react to an allergic challenge with blood or lung eosinophilia or hyperreactivity, compared to normal controls. Of interest, interleukin-5-knockout mice do not develop an enhanced *Mesocostoides corti* infection after exposure, as measured by the worm burden [20].

Clinical trials with humanized antibodies against interleukin-5 have begun. In the current trials these therapeutics inhibit eosinophilia in asthmatic persons, but an effect on lung function has yet to be established [21,22]. Further trials designed to measure eosinophil accumulation and lung function in asthmatic persons are currently underway, and will help to define the role of interleukin-5 and eosinophils in general in this disease.

Genomics and biochemistry of the interleukin-5 system

There are clusters of T-helper (Th)2-type cytokine genes, including that which encodes interleukin-5, on human chromosome 5q and in the mouse on chromosome 11q, indicating a common evolutionary origin [23]. The cDNA that encodes murine interleukin-5 was cloned in 1986 from a T-cell line, followed by isolation of interleukin-5 cDNA from a human T-cell leukemia line [24,25] using a murine interleukin-5 cDNA as a probe. No overall significant amino acid sequence homology was found to exist with other cytokines, except for short stretches in the murine interleukin-3, murine GM-CSF, and murine interferon- γ proteins [25]. Furthermore, in the interleukin-5 promoter region there are short stretches of conserved sequence motifs, designated CLE 0, CLE 1 and CLE 2, which are also found in the 5'-flanking regions of the interleukin-3, interleukin-4, and GM-CSF genes [23,26].

Biologically active interleukin-5 is a disulfide-linked homodimer that is held together by the highly conserved cysteine residues that orient the monomers in an antiparallel arrangement [27,28]. The higher homology of mouse and human interleukin-5 found in the carboxyl-terminal compared with the amino-terminal half is consistent with the binding site for the interleukin-5 receptor that resides between helices C and D at an arginine-rich region that comprises residues 89

through 92 [29–31]. The broad range of apparent molecular weights (45–60 kDa) of recombinant murine interleukin-5 and human interleukin-5 results from differential glycosylation, but deglycosylated interleukin-5 retains full biologic activity [32]. A crystal structure shows that human interleukin-5 is a novel two-domain configuration with each domain requiring the participation of two chains, with a high degree of similarity to the cytokine fold found in GM-CSF, interleukin-3, and interleukin-4 [33].

Like interleukin-4, interleukin-5 is produced by T cells that belong to the Th2 but not the Th1 subset. By virtue of the pattern of cytokines that they synthesize, Th2 cells are thought to control the growth and effector function of those cell types that are involved in allergic inflammatory responses [34–38]. As with other cytokines, regulation of interleukin-5 production is thought to result from activation of gene transcription [37]. Interleukin-5 synthesis is also regulated at the level of mRNA stability [39]. Interleukin-5 gene expression requires *de novo* protein synthesis, and is effectively inhibited by glucocorticoids and cyclosporine [36,37,40]. Furthermore, *in vivo* depletion of T cells in a mouse model of pulmonary inflammation reduces pulmonary eosinophilia, and interleukin-5 and other cytokine mRNA levels [38]. Mast cells and eosinophils also synthesize interleukin-5, indicating that autocrine production of interleukin-5 may contribute to the chronicity of inflammation [41,42].

The interleukin-5 receptor is in the type I cytokine family, which includes receptors for interleukin-2 through interleukin-7, GM-CSF, granulocyte-colony stimulating factor, and erythropoietin [10,43]. These receptors are integral membrane glycoproteins with amino-termini directed extracellularly, a single membrane-spanning domain, and several conserved features [10,43]. The human interleukin-5 receptor has a Kd of 170–330 pmol/l, and is expressed on eosinophils and eosinophilic sublines of the HL60 cell [44,45]. The high-affinity interleukin-5 receptor is composed of two noncovalently associated subunits: α and β . The 60 kDa human interleukin-5 receptor α -chain binds mouse and human interleukin-5 with relatively high affinity (Kd = 1 nmol/l) [46], but does not induce signal transduction. Interaction of the α -subunit/interleukin-5 complex with the β -subunit, which is shared with the GM-CSF receptor and the interleukin-3 receptor, increases affinity to approximately 250 pmol/l and facilitates functional activity [9]. A soluble receptor form of the interleukin-5 receptor α has been identified, which antagonizes both binding and function of interleukin-5, and may protect against excessive eosinophil recruitment and activation [9].

Protein tyrosine kinases that physically associate with cytokine receptors and become activated after ligand binding have been identified [47]. Utilizing the β -subunit, interleukin-3, GM-CSF and interleukin-5 primarily activate Janus kinase (JAK)2 in response to ligand–receptor

binding [47,48]. Activation of the JAK proteins is normally associated with autophosphorylation. Like interleukin-3 and GM-CSF, interleukin-5 induces rapid tyrosine phosphorylation of several proteins, further indicating that tyrosine kinases are involved in the cellular activation pathways [47,49]. JAK2 then induces tyrosine phosphorylation of STAT5, which activates its DNA-binding ability [47,49] and the ensuing cell activation [48].

Biology of interleukin-5

In the human, interleukin-5 is selective for eosinophils and basophils, whereas in the mouse it also acts on B lymphocytes [3,7,50]. Of course, eosinophils and basophils are two predominant effector cell types in allergic inflammation. By associating with its receptor, interleukin-5 effects eosinophil growth and differentiation [4,5,50,51], migration [8,50,52], activation and effector function [50,53,54], and survival [50,55]. As opposed to interleukin-3 or GM-CSF, only interleukin-5 promotes growth and differentiation to mature eosinophils in the bone marrow. Interleukin-3 and GM-CSF are also less selective than interleukin-5, stimulating the production of other granulocytes such as mast cells and neutrophils, respectively [50,56]. Because eosinophils are a dominant cell type in allergic reactions, this exquisite specificity makes interleukin-5 an excellent target for attenuating these responses. In fact, prolonged eosinophil survival and decreased eosinophil apoptosis caused by interleukin-5 are reversed by glucocorticoids [57,58], which accounts for at least some of the efficacy of these agents.

Activated eosinophils synthesize and release mediators, and secrete preformed granule constituents [59–62]. The eosinophil responds to a unique set of physiologic triggers, including secretory immunoglobulin A [59], which result largely from a Th2-type lymphocyte response. Eosinophils and neutrophils respond to many common stimulators, such as C5a, phorbol myristate acetate, zymosan, and formyl-methionyl-leucyl-phenylalanine [25, 60–65], with a respiratory burst, activation of phospholipases, production of eicosanoids, and secretion of preformed granule contents such as peroxidases and proteinases, including lysozyme and collagenases [63–65]. On the other hand, the ability to store and secrete the cationic low-molecular-weight proteins major basic protein, eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN) is unique to the eosinophil [66]. Major basic protein and ECP can lyse cells and can cause tissue damage at low concentrations [67–69]. Although EDN also has a pI of 11, it is not as innately toxic to tissue, indicating that there is more to this cytotoxicity than just the positive charge [67].

Animal models of interleukin-5 action

As a result of its efficacy and selectivity, interleukin-5 is an ideal drug development target for allergic eosinophil-

mediated diseases. With the development of neutralizing monoclonal antibodies to interleukin-5, interleukin-5-deficient mice, *in situ* hybridization methodology, and immunocytochemical techniques, it has been possible to investigate the role of interleukin-5 in allergic inflammatory responses in animals and humans.

Because the activity of interleukin-5 is restricted to eosinophils, it should be an ideal target to block this response in the lungs of allergic animal models of asthma, and has been studied in several species. Sensitized guinea pigs respond to allergic challenge with bronchial hyperresponsiveness and infiltration of eosinophils into lung tissue and bronchoalveolar lavage (BAL) fluid [11,13,70]. Monoclonal antibodies to interleukin-5 inhibit these pulmonary responses [13]. In contrast, larger doses of an anti-interleukin-5 antibody are needed to block the hyperreactivity than are needed to block the eosinophilia. This suggests either that interleukin-5 has effects on bronchoconstrictor reactivity that are independent of its effects on eosinophils, or that eosinophil activation, degranulation and release of its cytotoxic products, which were not measured in these studies, are the relevant aspects of eosinophil function that correlate with the development of the hyperreactivity. Indeed, it has been shown [71] that delivery of recombinant human interleukin-5 to the lungs of naïve guinea pigs increases eosinophils and neutrophils in the lungs and bronchoalveolar fluid, but this condition is not associated with augmented bronchoconstrictor responsiveness. However, recent studies have shown that administration of recombinant interleukin-5 to isolated airway smooth muscle from both rabbits and humans results in increased reactivity to acetylcholine [72]. In these studies the interleukin-5-induced hyperreactivity was abated by blocking the activity of interleukin-1, and interleukin-1 β mRNA and protein levels are increased by interleukin-5. Interleukin-5 may contribute to airway hyperreactivity by both indirect and direct mechanisms. In fact, it may work indirectly by releasing granule proteins from eosinophils that act as endogenous allosteric antagonists at inhibitory presynaptic muscarinic M₂ receptors, augmenting bronchoconstrictor responses to vagal nerve stimulation [73]. It may also work directly by mediating synthesis of interleukin-1 β in airway smooth muscle [72].

As with guinea pigs, antigen challenge to the lungs of sensitized mice causes an influx of eosinophils into the BAL fluid and lung tissue [74]. This effect is inhibited when monoclonal antibodies to interleukin-5 are given before the antigen challenge [75]. There is also increased expression of mRNA for Th2 cytokines such as interleukin-5 and interleukin-4 in the lungs of allergic mice [38]. To a lesser extent than are T lymphocytes, mast cells are involved in the development of pulmonary eosinophilia in allergic mice after single provocation by antigen [76], but are much less important in the pulmonary eosinophilia that occurs after a

multiple antigen challenge paradigm [77]. Mice have been developed using standard technology that are deficient in interleukin-5 [20]. These mice produce constitutive levels of eosinophils driven by other cytokines, and have normal circulating levels of immunoglobulin E, but do not mount an eosinophilic response to allergic challenge.

After multiple exposure to inhaled antigen, sensitized mice exhibit airway hyperreactivity [19,20]. When challenged in this manner, both the lung and lavage eosinophilia and the airway hyperreactivity to cholinergic agents are blocked by anti-interleukin-5 antibodies [20]. In addition, in sensitized interleukin-5-deficient mice receiving multiple challenges, the hyperreactivity is eliminated along with the airway eosinophilia [19,20]. In a variety of knockout and transgenic mice that were further modified by the administration of cytokines, chemokines or antibodies, there appear to be significant interactions among these proteins with regard to establishing eosinophilia and airways hyperreactivity [78]. Whereas interleukin-4 and interleukin-13 are redundant with regard to these inflammatory parameters, interleukin-5 plays a distinct role in both. Furthermore, interleukin-5 and eotaxin synergistically enhance eosinophilia and airway hyperreactivity in allergic mice by a CD4⁺ T-cell-dependent mechanism [79]. To some degree, these observations are dependent on the background strain of mouse [20,78].

Interleukin-5 has also been identified as an important cytokine in regulating human eosinophil survival in asthmatic persons after antigen challenge [57]. Inhibition of interleukin-5 activity during an established pulmonary eosinophilia could, therefore, cause tissue damage due to destruction of eosinophils and release of their inflammatory mediators. However, in allergic mice, administration of an antibody to interleukin-5 after antigen challenge, when lung eosinophilia was already established, did not increase tissue damage in the lungs [75]. These results have important therapeutic implications for the potential use of interleukin-5 inhibitors in the treatment of inflammatory airway disorders.

Allergic cynomolgus monkeys are also subject to an inflammatory cell influx into the airways, an early and late-phase bronchoconstriction, and bronchial hyperresponsiveness [14,80]. Treatment with a monoclonal antibody to interleukin-5 inhibits these responses to antigen provocation [14]. TRFK5, a monoclonal anti-interleukin-5 antibody, at an intravenous dose of 0.3 mg/kg inhibits lavage eosinophilia to 70%, while completely blocking the hyperreactivity to histamine. Furthermore, inhibition of both the pulmonary eosinophilia and bronchial hyperresponsiveness lasted for at least 3 months after a single treatment because of the long circulating lifetime of the antibody. Hence, in several animal models of asthma, blockade of interleukin-5 activity suppressed both eosinophilia and

changes in lung function, but the causal relationship between these two effects is somewhat tenuous.

Although there is often a correlation between lung eosinophilia, ECP in BAL fluid, and a decreased forced expiratory volume in 1 s (FEV₁) in humans [81], this does not necessarily indicate that the eosinophils are responsible for the decreased lung function. In fact, in several animal models there is a lack of correlation between reduced levels of lung eosinophils and improved lung function, suggesting that a critical activation step is missing [13–18]. In reality, there are no animal models that precisely duplicate the chronic nature of asthma.

Interleukin-5 in human asthma

Atopic asthmatic persons have increased expression of Th2-type cytokine (interleukin-2, interleukin-3, interleukin-4, interleukin-5, and GM-CSF) mRNA in both BAL fluid and in bronchial biopsies as compared with healthy volunteers, but there is no difference between the two groups in the expression of Th1-type cytokine mRNA such as interferon- γ [82–85]. The predominant source of interleukin-4 and interleukin-5 mRNA in asthmatic persons is the T lymphocyte, and the CD4⁺ and CD8⁺ T-cell populations express elevated levels of activation markers including interleukin-2 receptor (CD25), human leukocyte antigen-DR, and the very late activation antigen-1 [84,86–90]. These results suggest that atopic asthma is associated with activation of the interleukin-3, interleukin-4, interleukin-5, and GM-CSF gene cluster, a pattern that is consistent with a Th2-like T-lymphocyte response [85]. Interleukin-5 mRNA is also found in activated eosinophils and mast cells in tissues from patients with atopic dermatitis [91–93], allergic rhinitis [94,95], and asthma [82,89], raising the possibility that interleukin-5 arises from multiple sources in atopic individuals.

Eosinophil infiltration into the airways after allergen challenge is a consistent feature of atopic asthmatic persons [96–98]. Interleukin-5 is predominantly an eosinophil-active cytokine in the late-phase response to antigen challenge [99,100], and is important for the recruitment and survival of eosinophils [57,99]. On the other hand, interleukin-5 is probably not important in the acute response to allergen challenge in asthmatic persons. Indeed, interleukin-5 is not detectable in the BAL fluid of mildly asthmatic persons shortly after allergen provocation [100]. Interleukin-5 may also be important for the recruitment of eosinophils from blood vessels into tissues, because topical administration of recombinant human interleukin-5 to the nasal airway of persons with allergic rhinitis induced eosinophil accumulation into the nasal mucosa [101,102]. Interleukin-5 may also induce activation of eosinophils that are resident to inflamed tissue, but this effect may be secondary to activation of secretory immunoglobulin A [103].

Several studies have demonstrated a correlation between the activation of T lymphocytes, increased concentration of interleukin-5 in serum and BAL fluid, and increased severity of the asthmatic response [87,104–106]. In a study of 30 asthmatic persons, Robinson *et al* [86] found a strong correlation between the number of BAL cells that expressed mRNA for interleukin-5, the magnitude of baseline airflow obstruction (FEV₁), and bronchoconstrictor reactivity to methacholine. Furthermore, Zangrilli *et al* [106] found increased levels of interleukin-4 and interleukin-5 in the BAL fluid of asthmatic persons who had a late-phase response to antigen, but not in asthmatic persons who only demonstrated an early-phase response to antigen challenge. Motojima *et al* [104] compared serum levels of interleukin-5 in asthmatic patients during an exacerbation and in remission of asthma. Higher levels of serum interleukin-5 were found in each person during exacerbation, and patients with severe asthma had higher levels of serum interleukin-5 than did control individuals or patients with mild asthma. It is interesting to note that interleukin-5 levels were reduced in the serum of patients with moderate-to-severe asthma who were receiving oral glucocorticoids for control of their asthma [104,106]. These results are consistent with *in vitro* studies that show a potent inhibitory effect of corticosteroids on gene expression and on the release of pro-inflammatory cytokines, including interleukin-5, from inflammatory cells [107].

The link between interleukin-5, eosinophils, and asthma is currently under investigation using two humanized monoclonal antibodies specific for interleukin-5 that have been advanced into the clinic for evaluation as therapies for asthma. SCH55700 (reslizumab) is a humanized monoclonal antibody with activity against interleukin-5 from various species [108]. SB240563 (mepolizumab) is also a humanized antibody with specificity for human and primate interleukin-5 [109,110].

SCH55700 has an affinity for human interleukin-5 of 81 pmol/l and a 50% inhibitory concentration for inhibition of human interleukin-5-mediated TF-1 cell proliferation of 45 pmol/l. The efficacy of SCH55700 was further evaluated preclinically in a number of animal models. In a dose-dependent manner, SCH55700 inhibited total cell and eosinophil influx into BAL fluid, bronchi, and bronchioles of allergic mice for up to 8 weeks after a single 10 mg/kg dose and for 4 weeks after a single 2 mg/kg dose. Additional studies in allergic mice indicated that the combination of SCH55700 with an oral steroid (prednisolone) significantly increased the efficacy over that of either agent administered alone [108]. In allergic guinea pigs, SCH55700 caused a dose-dependent decrease in pulmonary eosinophilia and inhibited the development of allergen-induced airway hyperresponsiveness to substance P. It also inhibited the accumulation of total cells, eosinophils, and neutrophils in the lungs of guinea pigs

exposed to human interleukin-5. SCH55700 had no effect on the numbers of inflammatory cells in unchallenged animals or in animals challenged with GM-CSF, and had no effect on the levels of circulating total leukocytes [108]. In cynomolgus monkeys naturally allergic to *Ascaris suum*, postchallenge pulmonary eosinophilia was significantly decreased for up to 6 months after a single 0.3 mg/kg intravenous dose of SCH55700 [108].

A rising single-dose phase I clinical trial was conducted with SCH55700 in patients with severe persistent asthma who remained symptomatic despite intervention with high-dose inhaled or oral steroids [22]. The two highest doses of SCH55700 significantly decreased peripheral blood eosinophils, with inhibition lasting up to 90 days after the 1 mg/kg dose. There was also a trend toward improvement in lung function at the higher doses 30 days after dosing, with mean FEV₁ increasing by 11.2 and 8.6% in the 0.3 and 1.0 mg/kg groups, respectively, versus 4.0% in the placebo group [22].

Preclinical studies with SB240563 in cynomolgus monkeys indicated that peripheral blood eosinophils were decreased as a result of administration of the antibody [109,110]. Interestingly, maximal inhibition of peripheral blood eosinophils (80–90% of baseline) occurred 3–4 weeks after dosing (1 mg/kg subcutaneously), whereas maximal blood levels of the antibody were obtained 2–4 days after dosing, with a half-life of approximately 14 days.

SB240563 has also recently been tested in asthmatic persons in a clinical single-dose safety and activity study [21]. Patients with mild asthma were administered a single intravenous dose of SB240563 at either 2.5 or 10 mg/kg, or placebo. Patients were challenged with allergen 2 weeks before and 1 and 4 weeks after dosing. Peripheral blood and sputum eosinophil levels were measured, and early-phase and late-phase asthmatic responses were assessed by measuring the percentage fall in FEV₁ induced by allergen challenge. Both doses of SB240563 caused a significant reduction in peripheral blood eosinophils. Eosinophil counts were reduced in the 10 mg/kg dose group by approximately 75% for up to 16 weeks, and in the 2.5 mg/kg dose group by approximately 65% for up to 8 weeks. Postchallenge sputum eosinophils were also reduced in the 10 mg/kg dose group. Neither dose of SB240563 attenuated the fall in FEV₁ induced by allergen challenge in these mildly asthmatic persons.

With both of these antibodies showing acceptable safety profiles, larger studies can be conducted to determine the impact of blocking interleukin-5 on the pathophysiology of asthma and other eosinophil-related diseases. Only when these clinical trials are conducted will we be able to determine whether interleukin-5-based therapy in humans will

measure up to the promise that is projected from animal models.

Conclusion

There are circumstantial but compelling data that implicate interleukin-5 in diseases that involve eosinophils. Interleukin-5 is produced in lymphocytes, mast cells, eosinophils, and airway smooth muscle and epithelial cells, and is primarily responsible for the maturation and release of eosinophils in the bone marrow. In humans, it is a very selective cytokine because only eosinophils and basophils possess a type-1 cytokine receptor for interleukin-5 with a specific α -subunit and the β_c -subunit that confers high-affinity binding and signal transduction. A specific inhibitor of interleukin-5 could, therefore, attenuate pulmonary inflammation and the consequent pathophysiology without the potential for immunosuppressive side effects that exist with steroids.

Interleukin-5 in the circulation has been reduced by potent, neutralizing anti-interleukin-5 monoclonal antibodies. As a result, eosinophils have been attenuated for long durations in various animal models of eosinophil accumulation. In some but not all of these animal models, inhibition of tissue or BAL eosinophilia correlates with decreased pathophysiology. In addition, interleukin-5-knockout mice do not respond to an allergic challenge with blood or tissue eosinophilia. Furthermore, these mice are not overly sensitive to parasitic infection and, as opposed to their normal counterparts, are not hyperreactive to cholinergic challenge to the lungs. By contrast, although eosinophil levels were reduced by an anti-interleukin-5 antibody in a human allergic challenge model, there was no reduction in hyperreactivity. In a phase I clinical trial with another humanized anti-interleukin-5 antibody, eosinophils were reduced for 90 days in severe steroid-dependent asthmatic persons. Nevertheless, ongoing phase II studies are required to determine the utility of this approach in treating asthma and other eosinophilic diseases.

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